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Comparative activity of baculoviruses against the codling moth *Cydia pomonella* and three other tortricid pests of tree fruit

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Abstract

The granulovirus of *Cydia pomonella* (L.) (*Cp*GV) offers potential for selective control of codling moth. Two major limitations of *Cp*GV are its narrow host range and lack of persistence in the orchard agroecosystem. The nucleopolyhedroviruses of the alfalfa looper *Autographa californica* (Speyer) (*Ac*MNPV) and those of the celery looper *Anagrapha falcifera* (Kirby) (*Af*MNPV) have broad host ranges. Comparative assays of *Cp*GV, *Ac*MNPV, and *Af*MNPV against codling moth neonate larvae revealed a 54–93-fold greater susceptibility of codling moth to the granulovirus than to the two nucleopolyhedroviruses based on the LC_{50} values for each virus. The LC_{50}s for *Cp*GV, *Af*MNPV, and *Ac*MNPV were 32.7 capsules/mm^2, 1.77×10^3 occlusion bodies (OBs)/mm^2, and 3.05×10^3 OBs/mm^2, respectively. The LT_{50} determined for *Af*MNPV using an approximate LC_{95} of the virus against neonate larvae was 3.6 days. Histological examination of tissues in moribund codling moth larvae that had been treated with *Af*MNPV revealed the presence of nonoccluded and unenveloped virus rods in midgut tissue. Neither OBs nor signs of infection were detected in other tissues. The activity of *Af*MNPV was also evaluated in three other tortricid apple pests (obliquebanded leafroller, *Choristoneura rosaceana* (Harris); *Pandemis* leafroller, *Pandemis pyrusana* Kearfott; and the oriental fruit moth, *Grapholitha molesta* (Busck)). Codling and Oriental fruit moths were significantly more susceptible to *Af*MNPV than were the two leafroller species.

Keywords: *Cydia pomonella*; *Choristoneura rosaceana*; *Pandemis pyrusana*; *Grapholitha molesta*; Codling moth granulovirus; Nucleopolyhedrovirus; Baculovirus

1. Introduction

Several species of Lepidoptera in the family Tortricidae are principal pests of apple and pear production in the Pacific Northwest United States (Beers et al., 1993). The key insect pest in the region is the codling moth (CM), *Cydia pomonella* (L.). Control of CM has been traditionally accomplished with conventional chemical insecticides such as azinphos methyl (Guthion). Development of insecticide resistance and concerns over a safe food supply and environmental contamination necessitate the development of alternative interventions.

The granulovirus of CM (*Cp*GV) offers potential for the control of the moth without deleterious effects on the environment (Falcon et al., 1968; Huber and Dickler, 1977; Jaques et al., 1994; Lacey et al., 2000; Vail et al., 1991). Two major limitations of *Cp*GV are its narrow host range and lack of persistence in the orchard agroecosystem (Cross et al., 1999).

Two baculoviruses with broad host ranges within the Lepidoptera are the multiply enveloped nucleopolyhedroviruses of the alfalfa looper, *Autographa californica* (Speyer) (*Ac*MNPV) and those of the celery looper, *Anagrapha falcifera* (Kirby) (*Af*MNPV). The safety of baculoviruses in general and *Ac*MNPV in particular for vertebrates and invertebrate nontarget organisms is well documented (Gröner, 1990). A baculovirus with good entomopathogenic activity for CM, leafrollers, and other lepidopterous species would provide an alternative to broad spectrum insecticides without the disruption of natural enemy complexes that often occurs with conventional insecticides. In this paper, we present the

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results of research on susceptibility of CM to *Cp*GV, *Af*MNPV, and *Ac*MNPV and of three other tortricid pests of tree fruit to *Af*MNPV.

2. Materials and methods

2.1. Preparation of inoculum

Stocks of *Af*MNPV and *Ac*MNPV polyhedral occlusion bodies (OBs) used in bioassays were produced in *Trichoplusia ni* (Hübner) larvae using inoculum that had been stored frozen at the USDA-ARS laboratory in Fresno, CA, and procedures prescribed by Hostetter and Putler (1991). OBs were harvested and concentrated in deionized water according to methods described by Hostetter and Putler (1991). The concentration of OBs for each virus suspension was determined with a light microscope and a hemacytometer. One ml aliquots of the virus suspensions were placed in 1.5 ml Eppendorf vials and stored at −80 °C until used. The Carpovirusine formulation of *Cp*GV (6.7×10^8 virus capsules/ml; Calliope, Noguère, France) was used for comparative bioassays against CM neonates.

2.2. Bioassay procedure

Larvae used in bioassays were obtained from colonies maintained on artificial diet at the USDA-ARS Yakima Agricultural Research Laboratory and the Horticultural Crops Research Laboratory in Fresno, CA. Bioassays against neonate larvae of CM, obliquebanded leafroller (OBLR), *C. rosaceana* (Harris), the *Pandemis* leafroller, *Pandemis pyrusana* Kearfott, and the oriental fruit moth (OFM), *G. molesta* (Busck), were conducted on artificial diet in 2-ml plastic conical autosampler vials (Daigger, Lincolnshire, IL, USA). A 2-mm diameter hole in the cap of each vial covered with stainless steel screen (150 mesh) eliminated condensation. The codling moth diet described by Brinton et al. (1969) (BioServ, Frenchtown, NJ, USA) was used for CM and OFM neonate larvae. A pinto bean-based diet (Shorey and Hale, 1965) was used for assays with the two leafroller species. One ml molten diet was added to each vial and allowed to cool before application of viral suspension. Bioassays of *Cp*GV granules and *Af*MNPV and *Ac*MNPV OBs were performed with CM neonates using five concentrations of each virus that produced mortality ranging from 20 to 90%. Aliquots of *Af*MNPV and *Ac*MNPV were thawed at room temperature and diluted just prior to bioassay. The Carpovirusine formulation of *Cp*GV was stored at 2 °C and diluted just prior to use. Bioassays with *Af*MNPV were also performed against the other three tortricid species under the same conditions described for CM. Dosage varied depending on the target species and ranged from 3.98×10^2 to 1.99×10^4 OBs/mm^2 of diet surface. A 10 µl suspension of virus was added to each vial using a micropipette. Vials were then tilted and rotated to ensure even coverage of the surface. The treated vials were left uncapped for 2 h before adding a single neonate larva to each vial. Thirty neonates of each species were used for each concentration of virus and control. Larvae were incubated at 25 °C for 10 days before determination of mortality. Replicate tests were conducted on at least three separate dates.

Additionally, groups of 45 CM neonate larvae were exposed to an approximate LC_{95} dosage of *Af*MNPV (1.99×10^4 OBs/mm^2) in the manner prescribed above and monitored daily to track the progression of mortality over a 10 day observation period. Forty-five control larvae were monitored for mortality over the same period. The procedure was repeated on three separate dates. To test for mortality due to nonviral components of the MNPV preparations, lyophilized *Af*MNPV and *Ac*MNPV samples were inactivated by autoclaving (short cycle, 121 °C at 15 psi for 30 min) and each was bioassayed against 60 neonate CM larvae as described above. Concurrent bioassays using active virus and an untreated control were also conducted using 60 neonate larvae for each viral preparation and control. The bioassays were repeated on two separate dates.

2.3. Statistical analysis

Probit analysis of mortality data from bioassays of *Cp*GV, *Af*MNPV, and *Ac*MNPV against CM neonates and *Af*MNPV against the four tortricid species was conducted using Polo PC software (LeOra Software, 1987). Mortality data were corrected for control mortality using Abbott's formula. The LT_{50} values for *Af*MNPV in codling moth larvae were derived from analysis of data on the progression of mortality of neonate CM larvae, following exposure to the LC_{95} dosage of *Af*MNPV using probit analysis modified for multiple observations over time (Throne et al., 1995) and Mathematica software (Wolfram, Champaign, IL).

2.4. Histological examinations of codling moth

Groups of 20, third instar CM larvae were exposed to two times the neonate LC_{95} of either *Af*MNPV or *Ac*MNPV and monitored until the first signs of mortality. Twenty untreated larvae were used as controls. Moribund virus-treated and control larvae were fixed in alcoholic Bouin's solution for a minimum of 24 h and then dehydrated in a graded series of ethanol, infiltrated with xylene and paraffin, and embedded in Paraplast according to procedures outlined by Becnel (1997). The specimens were serially sectioned (6 µm thickness) with an AO Spencer microtome stained on microscope slides using the techniques described by Hamm (1966), and observed with a Zeiss (Axioskop 20) phase contrast photomicroscope fitted with an MC 80 camera. All tissues in 20 larvae treated with each virus were examined for signs of viral infection and production of OBs. Tissues of moribund CM larvae that had been treated with the *Af*MNPV were also dissected in 2.5% cold glutaraldehyde, prepared for electron microscopy using procedures described by Becnel (1997), and examined with a Siemens Elmiskop IA electron microscope.

3. Results

Comparative assays with *Cp*GV, *Af*MNPV, and *Ac*MNPV reveal a 54–93-fold greater susceptibility of CM to the granulovirus than to the two nucleopolyhedroviruses based on the LC_{50}s for each virus. (Table 1). Although the LC_{50} of *Af*MNPV was 1.7-fold lower than that of *Ac*MNPV, there was substantial overlap in the 95% confidence limits of the two viruses. The LC_{95} values *Af*MNPV and *Ac*MNPV were 16 and 25 times greater, respectively, than that of *Cp*GV. Mortality of CM larvae exposed to inactivated virus was not significantly different from that of controls. The mean mortalities ($\pm$SE) for CM larvae exposed to inactivated *Ac*MNPV and *Af*MNPV and untreated controls were 2.35 ± 0.65, 4.35 ± 2.65, and 4.0 ± 4.0, respectively. The active *Ac*MNPV and *Af*MNPV preparations produced mean mortalities ($\pm$SE) of 86.0 ± 1.0 and 77.5 ± 2.5, respectively. The progressions of mortality in CM larvae over a 10 day period following exposure to an approximate LC_{95} dosage of *Af*MNPV are presented in Fig. 1. The LT_{50} produced at the approximate LC_{95} dosage was 3.6 d (95% c.i. 2.5–5.2 days; $\chi^2 = 23.18$, df $= 8$; heterogeneity $= 2.58$; slope $= 2.11$ SE of slope $= 0.16$).

In comparative assays with *Af*MNPV against the 4 tortricids, CM and OFM were significantly more susceptible to the virus than the two leafrollers based on nonoverlap of 95% confidence limits of LC_{50} values (Table 2). Despite overlap of the 95% confidence intervals of the two leafrollers, the LC_{50} of the *Pandemis* leafroller was nearly half of that of OBLR.

Table 1
Comparative susceptibility of neonate codling moth larvae to the granulovirus of *C. pomonella* (*Cp*GV) and the nucleopolyhedroviruses of *A. falcifera* (*Af*MNPV) and *A. californica* (*Ac*MNPV)[a]

Virus	Occlusion bodies/mm² (95% confidence limit)	
	LC_{50}	LC_{95}
*Cp*GV	32.69	1.03×10^3
	$(1.66 \times 10–6.35 \times 10)$	$(3.06 \times 10^2–2.82 \times 10^4)$
*Af*MNPV	1.77×10^3	1.67×10^4
	$(1.19 \times 10^3–2.52 \times 10^3)$	$(9.02 \times 10^3–5.53 \times 10^4)$
*Ac*MNPV	3.05×10^3	2.55×10^4
	$(5.73 \times 10^2–5.67 \times 10^3)$	$(1.25 \times 10^4–2.78 \times 10^5)$

[a] Mean control mortality was 10.2%.

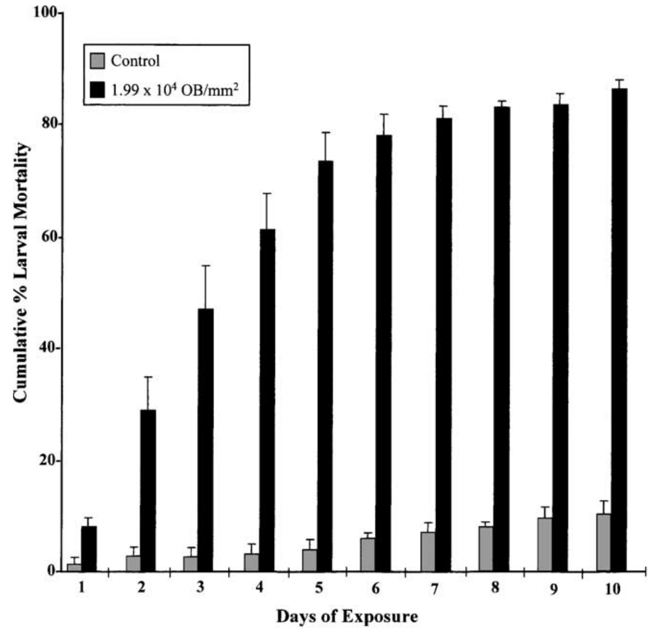

Fig. 1. Progression of mortality after exposure of codling moth neonate larvae to an approximate LC_{95} dosage of the *A. falcifera* multi-enveloped nucleopolyhedrovirus.

Table 2
Comparative susceptibility of neonate larvae of *C. pomonella* and three other tortricid fruit pests to the nucleopolyhedrovirus of *A. falcifera*

Species/dosage	Occlusion bodies/mm² (95% confidence limit)	
	LC_{50}	LC_{95}
C. pomonella	1.77×10^3	1.67×10^4
	$(1.19 \times 10^3–2.52 \times 10^3)$	$(9.02 \times 10^3–5.53 \times 10^4)$
P. pyrusana	4.30×10^3	3.19×10^4
	$(3.53 \times 10^3–5.32 \times 10^3)$	$(2.14 \times 10^4–5.64 \times 10^4)$
C. rosaceana	7.63×10^3	8.45×10^4
	$(4.47 \times 10^3–1.78 \times 10^4)$	$(2.97 \times 10^4–1.35 \times 10^6)$
G. molesta	2.06×10^3	9.55×10^3
	$(1.30 \times 10^3–2.91 \times 10^3)$	$(5.48 \times 10^3–5.15 \times 10^4)$

Histological examination of larvae that had been treated with *Af*MNPV and *Ac*MNPV revealed a lack of OBs in any tissues. Typically, large OBs (2.5 μm diameter) are observed in the nuclei of a wide range of tissues in noctuid and pyralid hosts. Symptoms that are associated with infection of noctuid hosts by *Af*MNPV and *Ac*MNPV (i.e., liquification of tissues) were also lacking in CM larvae. Nonenveloped viral rods were observed in electron micrographs of midgut columnar epithelial tissue, but not in other tissues.

4. Discussion

The susceptibility of neonate larvae to the Carpovirusine formulation of *Cp*GV reported in our study was comparable to that observed by Sheppard and Stairs (1977). They reported an LC_{50} of 5 capsules/larva based on the portion of treated surface that was consumed. Although the LC_{50} of *Cp*GV in our study was 33 capsules/mm^2, neonate larvae consumed less than 1 mm^2 of the treated surface before burrowing into untreated medium.

The difference in susceptibility of CM larvae to *Cp*GV and the two MNPVs is even greater than what the data in Table 1 indicate. The number of virions contained in occlusion bodies of MNPVs can approach 200/OB (Ackermann and Smirnoff, 1983). Volkman and Summers (1977) estimated the number of virions per OB of *Ac*MNPV at 190. Whereas, capsules of *Cp*GV and other granuloviruses characteristically contain a single virion. The differences in susceptibility of CM and OFM and the two leafroller species to *Af*MNPV are probably greater than those shown in Table 2. Both CM and OFM bore through the surface of the medium and host fruits, but the leafrollers browse the surface consuming more OBs. The method of surface treatment rather than droplet assays or homogeneously treated diet for our tests was used specifically because of the feeding habits of the four species in nature.

The lack of viral development of the MNPVs beyond the midgut tissue of CM helps to explain the gross difference in virulence between *Cp*GV and the two MNPVs. A broad range of host tissues are infected by *Ac*MNPV and *Af*MNPV in noctuid hosts (Vail et al., 1999; Vail and Jay, 1973). A similar range of tissues in the pyralids *Cadra figulilella* (Gregson) and *Amyelois transitella* (Walker) are infected by *Af*MNPV (Cardenas et al., 1997; Vail et al., 1993). In CM, the unenveloped virus rods of *Af*MNPV were ostensibly unable to pass through midgut tissue, which is required for infection of other host tissues (Federici, 1997).

Numerous lepidopteran species in several families are orchard pests worldwide. When some key pests are controlled using specific alternative interventions, secondary pests may emerge as a problem. For example, in the western United States, secondary leafroller pests are incidentally controlled by application of the broad spectrum organophosphate, azinphos methyl, for CM control. When specific control of CM is accomplished with pheromones that disrupt mating, leafrollers may reach economically important levels (Walker and Welter, 2001). Similarly, *Cp*GV will specifically control CM, but not affect other pest lepidopterans. Several other viruses have also been reported from tortricid orchard pests and related species, but most are specific to one host species (Cross et al., 1999; Lacey et al., 2000). On the other hand, both MNPVs we tested have very broad host ranges.

*Af*MNPV has been reported to infect over 31 species of Lepidoptera in 10 families (Hostetter and Putler, 1991; Vail et al., 1999). The host range of *Ac*MNPV is also broad including 33 species of Lepidoptera in 12 families (Vail et al., 1999). The virulence of both viruses for species in the Noctuidae is especially high. Vail et al. (1970, 1978) report 10-day post-exposure LC_{50} values for *Ac*MNPV against *Trichoplusia ni* (Hübner), *Heliothis virescens* (Fabricius), *Helicoverpa zea* (Boddie), and *Spodoptera exigua* (Hübner) of 0.23, 0.28, 13.99, and 3.64 OBs/mm^2, respectively. Seven-day post-exposure values for *Af*MNPV against the same species are 0.093, 0.095, 0.147, and 0.327 OBs/mm^2, respectively (Vail et al., 1996). Genetic evidence presented by Harrison and Bonning (1999) indicates that *Af*MNPV and the nucleopolyhedrovirus of *Rachiplusia ou* (Guenee) (*Ro*MNPV) are the same virus. Also their bioassays against *Ostrinia nubilalis* (Hübner), *H. virescens*, and *H. zea* failed to detect any differences in the larvicidal activities of *Ro*MNPV and *Af*MNPV.

According to van Beek and Hughes (1998), virulence of baculoviruses is best determined by the speed with which a given virus elicits the desired response. In the case of *Ac*MNPV and *Af*MNPV and activity against tortricid fruit tree pests, dosage also has to be taken into account. Although *Af*MNPV and *Ac*MNPV are far less active for CM than *Cp*GV, their host spectra are considerably wider. The potential for increasing virulence and reducing the time required to kill other target insects has been demonstrated for *Ac*MNPV after the incorporation of genes for insect-specific toxins into the viral genome or other genetic modifications (Hughes et al., 1997; Wood and Hughes, 1996). Although the dosages that produced 95% larval mortality in our study are prohibitive in terms of economical control, our results provide a baseline from which to measure future improvements in virulence. If a marked increase in virulence of these baculoviruses toward CM and other tortricids is possible, the benefits of applying a single virus against a broad range of insects could facilitate practical use of these viruses for control of tortricids and other lepidopteran pests of tree fruit.

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